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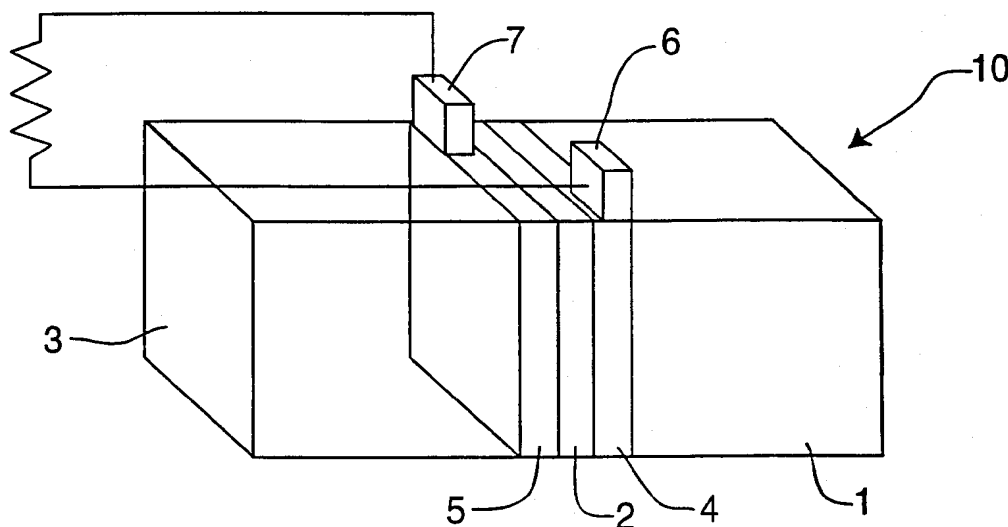
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## (57) Abstract

Provided is a battery comprising a first compartment, a second compartment and a barrier separating the first and second compartments, wherein the barrier comprises a proton transporting moiety.

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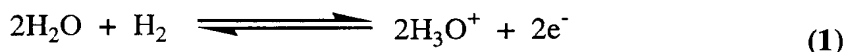
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## ENZYMATIC BATTERY

The present invention relates to batteries, including fuel cells and re-chargeable fuel cells, for use in powering electrical devices.

5 Batteries such as fuel cells are useful for the direct conversion of chemical energy into electrical energy. Fuel cells are typically made up of three chambers separated by two porous electrodes. A fuel chamber serves to introduce a fuel, typically hydrogen gas, which can be generated *in situ* by "reforming" hydrocarbons such as methane with steam, so that the hydrogen contacts H<sub>2</sub>O at the first electrode, where, when a circuit is formed between the  
10 electrodes, a reaction producing electrons and hydronium (H<sub>3</sub>O<sup>+</sup>) ions is catalyzed.



A central chamber can comprise an electrolyte. The central chamber acts to convey hydronium ions from the first electrode to the second electrode. The second electrode provides an interface with a recipient molecule, typically oxygen, found in the third chamber.  
15 The recipient molecule receives the electrons conveyed by the circuit.



The electrolyte element of the fuel cell can be, for example, a conductive polymer material such as a hydrated polymer containing sulfonic acid groups on perfluoroethylene side chains on a perfluoroethylene backbone such as Nafion<sup>TM</sup> (du Pont de Nemours, Wilmington,  
20 DE) or like polymers available from Dow Chemical Co., Midland, MI. Other electrolytes include alkaline solutions (such as 35 wt %, 50 wt % or 85 wt % KOH), acid solutions (such as concentrated phosphoric acid), molten electrolytes (such as molten metal carbonate), and solid electrolytes (such as solid oxides such as yttria (Y<sub>2</sub>O<sub>3</sub>)-stabilized zirconia (ZrO<sub>2</sub>)). Liquid electrolytes are often retained in a porous matrix. Such fuel cells are described, for  
25 example, in "Fuel Cells," *Kirk-Othmer Encyclopedia of Chemical Technology*, Fourth Edition, Vol. 11, pp. 1098-1121.

These types of fuel cells typically operate at temperatures from about 80°C to about 1,000°C. The shortcomings of the technology include short operational lifetimes due to catalyst poisoning from contaminants, high initial costs, and the practical restrictions on  
30 devices that operate at relatively high to extremely high temperatures.

The present invention provides a fuel cell technology that employs molecules used in biological processes to create fuel cells that operate at moderate temperatures and without the presence of harsh chemicals maintained at high temperatures, which can lead to corrosion of the cell components. While the fuel used in the fuel cells of the invention are more complex, they are readily available and suitably priced for a number of applications, such as power supplies for mobile computing or telephone devices. It is anticipated that fuel cells of the invention can be configured such that a 300 cc cell has a capacity of as much as 80 W•h - and thus can have more capacity than a comparably sized battery for a laptop computer – and that such cells could have still greater capacity. Thus, it is believed that the fuel cells of the invention can be used to increase capacity, and/or decrease size and/or weight. Moreover, the compact, inert energy sources of the invention can be used to provide short duration electrical output. Since the materials retained within the fuel cells are non-corrosive and typically not otherwise hazardous, it is practical to recharge the fuel cells with fuel, with the recharging done by the consumer or through a service such as a mail order service.

Moreover, in certain aspects, the invention provides fuel cells that use active transport of protons to increase sustainable efficiency. Fuel cells of the invention can also be electrically re-charged.

#### **SUMMARY OF THE INVENTION**

In one aspect, the invention provides a fuel cell comprising a first compartment, a second compartment and a barrier separating the first and second compartments, wherein the barrier comprises a proton transporting moiety.

In another aspect, the invention provides a fuel cell a first compartment; a second compartment; a barrier separating the first compartment from the second compartment; a first electrode; a second electrode; a redox enzyme in the first compartment in communication with the first electrode to receive electrons therefrom, the redox enzyme incorporated in a lipid composition; an electron carrier in the first compartment in chemical communication with the redox enzyme; and an electron receiving composition in the second compartment in chemical communication with the second electrode, wherein, in operation, an electrical current flows along a conductive pathway formed between the first electrode and the second electrode.

**BRIEF DESCRIPTION OF THE DRAWING**

**Figure 1** displays a perspective view of the interior of a fuel cell with three chambers.

**Figures 2** illustrates a fuel cell exhibiting certain preferred aspects of the present invention.

5        **Figure 3A, 3B and 3C** illustrate a similar fuel cell with scavenger-containing segment.

**Figures 4A and 4B** show a top view of a fuel cell with two chambers.

**Figure 5A** shows a top view of a fuel cell with two chambers, while **Figure 5B** shows a side view.

10       **Figure 6** shows a fuel cell where the fluids bathing the two electrodes are segregated.

**Figure 7** shows a fuel cell with incorporated light regulation and a sensor.

**DEFINITIONS**

15       The following terms shall have, for the purposes of this application, the respective meaning set forth below.

• **electron carrier:** An electron carrier is a composition that provides electrons in an enzymatic reaction. Electron carriers include, without limitation, reduced nicotinamide adenine dinucleotide (denoted NADH; oxidized form denoted NAD or NAD<sup>+</sup>), reduced nicotinamide adenine dinucleotide phosphate (denoted NADPH; oxidized form denoted NADP or NADP<sup>+</sup>), reduced nicotinamide mononucleotide (NMNH; oxidized form NMN), reduced flavin adenine dinucleotide (FADH<sub>2</sub>; oxidized form FAD), reduced flavin mononucleotide (FMNH<sub>2</sub>; oxidized form FMN), reduced coenzyme A, and the like. Electron carriers include proteins with incorporated electron-donating prosthetic groups, such as coenzyme A, protoporphyrin IX, vitamin B12, and the like. Further electron carriers include glucose (oxidized form: gluconic acid), alcohols (e.g., oxidized form: ethylaldehyde), and the like. Preferably the electron carrier is present in a concentration of 1 M or more, more preferably 1.5 M or more, yet more preferably 2 M or more.

• **electron-receiving composition:** An electron-receiving composition receives the electrons conveyed to the cathode by the fuel cell.

30    • **electron transfer mediator:** An electron transfer mediator is a composition which facilitates transfer to an electrode of electrons released from an electron carrier.

- **redox enzyme:** An redox enzyme is one that catalyzes the transfer of electrons from an electron carrier to another composition, or from another composition to the oxidized form of an electron carrier. Examples of appropriate classes of redox enzymes include: oxidases, dehydrogenases, reductases and oxidoreductases. Additionally, other enzymes, will redox catalysis as their secondary property could also be used e.g., superoxide dismutase.
- **composition.** Composition refers to a molecule, compound, charged species, salt, polymer, or other combination or mixture of chemical entities.

### Detailed Description

10        **Figure 1** illustrates features of an exemplary battery such as a fuel cell **10**. The fuel cell **10** has a first chamber **1** containing an electron carrier, with the textured background fill of the first chamber **1** illustrating that the solution can be retained within a porous matrix (including a membrane). Second chamber **2** similarly contains an electrolyte (and can be the same material as found in the first chamber) in a space, which space can also be filled with a  
15 retaining matrix, intervening between porous first electrode **4** and porous second electrode **5**. A face of second electrode **5** contacts the space of third chamber **3**, into which an electron receiving molecule, typically a gaseous molecule such as oxygen, is introduced. First electrical contact **6** and second electrical contact **7** allow a circuit to be formed between the two electrodes.

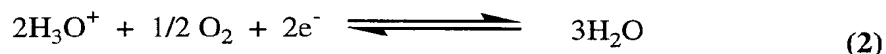
20        The optional porous retaining matrix can help retain solution in, for example, the second chamber **2** and minimize solution spillover into the third chamber **3**, thereby maintaining a surface area of contact between the electron receiving molecule and the second electrode **5**. In some embodiments, the aqueous liquid in the first chamber **1** and second chamber **2** suspends non-dissolved reduced electron carrier, thereby increasing the reservoir  
25 of reduced electron carrier available for use to supply electrons to the first electrode **4**. In another example, where the chambers include a porous matrix, a saturated solution can be introduced, and the temperature reduced to precipitate reduced electron carrier within the pores of the matrix. Following precipitation, the solution phase can be replaced with another concentrated solution, thereby increasing the amount of electron carrier, which electron  
30 carrier is in both solid and solvated form.

It will be recognized that the second chamber can be made up of a polymer electrolyte, such as one of those described above.

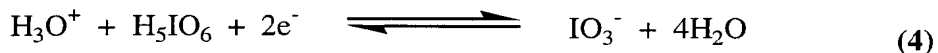
The reaction that occurs at the first electrode can be exemplified with NADH as follows:



Preferred enzymes relay the electrons to mediators that convey the electrons to the anode electrode. Thus, if the enzyme normally conveys the electrons to reduce a small molecule, this small molecule is preferably bypassed. The corresponding reaction at the second electrode is:



Using reaction 2, preferably the bathing solution is buffered to account for the consumption of hydrogen ions, or hydrogen ion donating compounds must be supplied during operation of the fuel cell. This accounting for hydrogen ion consumption helps maintain the pH at a value that allows a useful amount of redox enzymatic activity. To avoid this issue, an alternate electron receiving molecule with an appropriate oxidation/reduction potential can be used. For instance, periodic acid can be used as follows:



The use of this reaction at the cathode results in a net production of water, which, if significant, can be dealt with, for example, by providing for space for overflow liquid. Such alternative electron receiving molecules are often solids at operating temperatures or solutes in a carrier liquid, in which case the third chamber **3** should be adapted to carry such non-gaseous material. Where, as with periodic acid, the electron receiving molecule can damage the enzyme catalyzing the electron releasing reaction, the second chamber **2** can have a segment, as illustrated as item **8** in fuel cell **10'** of **Figure 2**, containing a scavenger for such electron receiving molecule.

In a preferred embodiment, the electrodes comprise metallizations on each side of a non-conductive substrate. For example, in **Figure 3A** the metallization on a first side of dielectric substrate **42** is the first electrode **44**, while the metallization on the second side is the second electrode **45**. Perforations **49** function as the conduit between the anode and cathode of the fuel cell, as discussed further below. The illustration of **Figure 3A**, it will be recognized, is illustrative of the relative geometry of this embodiment. The thickness of dielectric substrate **42** is, for example, from 15 micrometer ( $\mu\text{m}$ ) to 50 micrometer, or from 15 micrometer to 30 micrometer. The width of the perforations is, for example, from 20

micrometer to 80 micrometer. Preferably, perforations comprise in excess of 50% of the area of any area of the dielectric substrate involved in transport between the chambers, such as from 50 to 75% of the area. In certain preferred embodiments, the dielectric substrate is glass or an polymer, such as polyvinyl acetate or soda lime silicate.

5           **Fig. 3B** illustrates the electrodes framed on a perforated substrate in more detail. The perforations **49** together with the dielectric substrate **42** provide a support for lipid bilayers (i.e., membranes) spanning the perforations. Such lipid bilayers can incorporate at least a first enzyme or enzyme complex (hereafter "first enzyme") **62** effective (i) to oxidize the reduced form of an electron carrier, and preferably (ii) to transport, in conjunction with the oxidation,  
10       protons from the fuel side **41** to the product side **43** of the fuel cell **50**. Preferably, the first enzyme **62** is immobilized in the lipid bilayer with the appropriate orientation to allow access of the catalytic site for the oxidative reaction to the fuel side and asymmetric pumping of protons. However, as the fuel is substantially isolated on the fuel side **41**, an enzyme inserted into the lipid bilayer with the opposite orientation is without an energy source.

15           Examples of particularly preferred enzymes providing one or both of the oxidation/reduction and proton pumping functions include, for example, NADH dehydrogenase (e.g., from *E.coli*. Tran et al., "Requirement for the proton pumping NADH dehydrogenase I of *Escherichia coli* in respiration of NADH to fumarate and its bioenergetic implications," *Eur. J. Biochem.* 244: 155, 1997), NADPH transhydrogenase, proton ATPase,  
20       and cytochrome oxidase and its various forms. Methods of isolating such an NADH dehydrogenase enzyme are described in detail, for example, in Braun et al., *Biochemistry* 37: 1861-1867, 1998; and Bergsma et al., "Purification and characterization of NADH dehydrogenase from *Bacillus subtilis*," *Eur. J. Biochem.* 128: 151-157, 1982. The lipid bilayer can be formed across the perforations **49** and enzyme incorporated therein by, for  
25       example, the methods described in detail in Niki et al., US Patent 4,541,908 (annealing cytochrome C to an electrode) and Persson et al., *J. Electroanalytical Chem.* 292: 115, 1990. Such methods can comprise the steps of: making an appropriate solution of lipid and enzyme, where the enzyme may be supplied to the mixture in a solution stabilized with a detergent; and, once an appropriate solution of lipid and enzyme is made, the perforated dielectric  
30       substrate is dipped into the solution to form the enzyme-containing lipid bilayers. Sonication or detergent dilution may be required to facilitate enzyme incorporation into the bilayer. *See, for example*, Singer, *Biochemical Pharmacology* 31: 527-534, 1982; Madden, "Current



concepts in membrane protein reconstitution,” *Chem. Phys. Lipids* 40: 207-222, 1986; Montal et al., “Functional reassembly of membrane proteins in planar lipid bilayers,” *Quart. Rev. Biophys.* 14: 1-79, 1981; Helenius et al., “Asymmetric and symmetric membrane reconstitution by detergent elimination,” *Eur. J. Biochem.* 116: 27-31, 1981; Volumes on  
5 biomembranes (e.g., Fleischer and Packer (eds.)), in *Methods in Enzymology* series, Academic Press.

Using enzymes having both the oxidation/reduction and proton pumping functions, and which consume electron carrier, the acidification of the fuel side caused by the consumption of electron carrier is substantially offset by the export of protons. Net proton  
10 pumping in conjunction with reduction of an electron carrier can exceed 2 protons per electron transfer (e.g., up to 3 to 4 protons per electron transfer). Accordingly, in some embodiments care must be taken to buffer or accommodate excess de-acidification on the fuel side or excess acidification of the product side. Alternatively, the rate of transport is adjusted by incorporating a mix of redox enzymes, some portion of which enzymes do not exhibit  
15 coordinate proton transport. In some embodiments, care is taken especially on the fuel side to moderate proton export to match proton production. Acidification or de-acidification on one side or another of the fuel cell can also be moderated by selecting or mixing redox enzymes to provide a desired amount of proton production. Of course, proton export from the fuel side is to a certain degree self-limiting, such that in some embodiments the theoretical concern for  
20 excess pumping to the product side is of, at best, limited consequence. For example, mitochondrial matrix proteins which oxidize electron carriers and transport protons operate to create a substantial pH gradient across the inner mitochondrial membrane, and are designed to operate as pumping creates a relatively high pH such as pH 8 or higher. (In some  
embodiments, however, care is taken to keep the pH in a range closer to pH 7.4, where many  
25 electron carriers such as NADH are more stable.) Irrespective of how perfectly proton production is matched to proton consumption, the proton pumping provided by this embodiment of the invention helps diminish losses in the electron transfer rate due to a shortfall of protons on the product side.

In some embodiments, proton pumping is provided by a light-driven proton pump  
30 such as bacteriorhodopsin. Recombinant production of bacteriorhodopsin is described, for example, in Nassal et al., *J. Biol. Chem.* 262: 9264-70, 1987. All *trans* retinal is associated with bacteriorhodopsin to provide the light-absorbing chromophore. Light to power this type

of proton pump can be provided by electronic light sources, such as LEDs, incorporated into the fuel cell and powered by a (i) portion of energy produced from the fuel cell, or (ii) a translucent portion of the fuel cell casing that allows light from room lighting or sunlight to impinge the lipid bilayer. For example, illustrated in **Fig. 7** is a fuel cell **400** in which light control devices **71** are incorporated. These light control devices **71** contain, for example, LEDs or liquid crystal shutters. Liquid crystal shutters have a relatively opaque and a relatively translucent state and can be electronically switched between the two states. An external light source, such as the light provided by room lighting or sunlight can be regulated through the use of liquid crystal shutters or other shuttering device. In some embodiments, the light control devices are individually regulated or regulated in groups to aid in regulating the amount of light conveyed to the proton pump protein. Preferably, the light control devices **71** have lenses to direct the light to focus primarily at the dielectric substrate **42**, particularly those portions containing lipid bilayers incorporating the proton pumps. A monitoring device **72** can operate to monitor a condition in the fuel cell, such as the pH or the concentration of electron carrier, and relay information to a controller **73** which operates to moderate an aspect of the operation of the fuel cell should monitored values dictate such action. For example, the controller **73** can moderate the level of light conveyed by the light control devices **71** depending upon the pH of the fuel side **41**. Note that in one embodiment an external light source is allowed to energize the proton pump without the use of any light-regulating devices.

In another embodiment, redox enzyme is deposited on or adjacent to the first electrode, while a proton transporter is incorporated into the lipid bilayers of the perforations.

In another embodiment, a second enzyme **63** is incorporated into the fuel cell, such as into the lipid bilayer or otherwise on the first electrode or in the first chamber, to facilitate proton transport or generation in the first chamber during recharge mode, thereby adding protons to the fuel side. The second enzyme can be the same as, or distinct from, the enzyme that transports protons during forward operation. An example of this second enzyme include transporting proteins with lower redox potential relative to, for example, NAD succinate dehydrogenase in conjunction with the CoQH<sub>2</sub>-cyt c reductase complex. Also useful are lactate dehydrogenase and malate dehydrogenase, both enzymes isolated from various sources available from Sigma Chemical Co., St. Louis, MO. For example, bacteriorhodopsin can also be used with an orientation appropriate for this use in the recharge mode.

In some embodiments, the recharge mode operates to regenerate NADH, but does not reverse pump protons.

The perforations 49 are illustrated as openings. However, these can also comprise porous segments of the dielectric substrate 42. Alternatively, these can comprise membranes spanning the perforations 49 to support the lipid bilayer. Preferably, the perforations encompass a substantial portion of the surface area of the dielectric substrate, such as 50%. Preferably, enzyme density in the lipid bilayer is high, such as  $2 \times 10^{12}/\text{mm}^2$ .

The orientation of enzyme in the lipid bilayer can be random, with effectiveness of proton pumping dictated by the asymmetric presence of substrate such as protons and electron carrier. Alternatively, orientation is established for example by using antibodies to the enzyme present on one side of the membrane during formation of the enzyme-lipid bilayer complex.

The perforations 49 and metallized surfaces (first electrode 44 and second electrode 45) of the dielectric substrate 42 can be constructed, for example, with masking and etching techniques of photolithography well known in the art. Alternatively, the metallized surfaces (electrodes can be formed for example by (1) thin film deposition through a mask, (2) applying a blanket coat of metallization by thin film then photo-defining, selectively etching a pattern into the metallization, or (3) Photo-defining the metallization pattern directly without etching using a metal impregnated resist (DuPont Fodel process, *see*, Drozdyk et. al. "Photopatternable Conductor tapes for PDP applications" Society for Information Display 1999 Digest, 1044-1047; Nebe et al., US Patent 5,049,480). In one embodiment, the dielectric substrate is a film. For example, the dielectric can be a porous film that is rendered non-permeable outside the "perforations" by the metallizations. The surfaces of the metal layers can be modified with other metals, for instance by electroplating. Such electroplatings can be, for example, with chromium, gold, silver, platinum, palladium, nickel, mixtures thereof, or the like, preferably gold and platinum. In addition to metallized surfaces, the electrodes can be formed by other appropriate conductive materials, which materials can be surface modified. For example, the electrodes can be formed of carbon (graphite), which can be applied to the dielectric substrate by electron beam evaporation, chemical vapor deposition or pyrolysis. Preferably, surfaces to be metallized are solvent cleaned and oxygen plasma ashed.

As illustrated in **Figure 3C**, electrical contact **54** connects the first electrode **44** to a prospective electrical circuit, while electrical contact **55** connects the second electrode **45**.

In one embodiment, the product side of the fuel cell is comprised of an aqueous liquid with dissolved oxygen. In an embodiment, at least a portion of the wall retaining such aqueous liquid is oxygen permeable, but sufficiently resists transmission of water vapor to allow a useful product lifetime with the aqueous liquid retained in the fuel cell. An example of an appropriate polymeric wall material is an oxygen permeable plastic. In contrast, the fuel side is preferably constructed of material that resists the incursion of oxygen. The fuel cell can be made anaerobic by flushing to purge oxygen with an inert gas such as nitrogen or helium. In some rechargeable embodiments, the electron-receiving composition is regenerated during recharging mode, thereby eliminating or reducing the need for an outside supply of such electron-receiving composition.

The fuel cell of the invention can preferably be recharged by applying an appropriate voltage to inject electrons into the fuel side to allow the first enzyme to catalyze the reverse reaction. In particularly preferred embodiments, the first enzyme has both the oxidation/reduction and proton pumping functions and operates to reverse pump protons from the product side to the fuel side during recharging. Thus, the reverse pumping supplies the protons consumed in generating, for example, NADH from (i)  $\text{NAD}^+$  and (ii) the injected electrons and protons. Note that in reverse operation the injected electrons act first to reduce any oxygen resident in the fuel side, as this reaction is energetically favored. Once any such oxygen is consumed, the electrons can contribute to regenerating the reduced electron carrier.

The above discussion of the embodiments using proton transport focus on the use of both faces of a substrate to provide the electrodes, thereby facilitating a more immediate transfer of protons to the product side where the protons are consumed in reducing the electron-receiving composition. However, it will be recognized that in this embodiment structures such as a porous matrix can be interposed between the fuel side and the product side. Such an intervening structure can operate to provide temperature shielding or scavenger molecules that protect, for example, the enzymes from reactive compounds.

The fuel cell operates within a temperature range appropriate for the operation of the redox enzyme. This temperature range typically varies with the stability of the enzyme, and the source of the enzyme. To increase the appropriate temperature range, one can select the appropriate redox enzyme from a thermophilic organism, such as a microorganism isolated

from a volcanic vent or hot spring. Nonetheless, preferred temperatures of operation of at least the first electrode are about 80°C or less, preferably 60°C or less, more preferably 40°C or 30°C or less. The porous matrix is, for example, made up of inert fibers such as asbestos, sintered materials such as sintered glass or beads of inert material.

5           The first electrode (anode) can be coated with an electron transfer mediator such as an organometallic compound which functions as a substitute electron recipient for the biological substrate of the redox enzyme. Similarly, the lipid bilayer of the embodiment of **Fig. 3** or structures adjacent to the bilayer can incorporate such electron transfer mediators. Such organometallic compounds can include, without limitation, dicyclopentadienyliron ( $C_{10}H_{10}Fe$ ,  
10   ferrocene), available along with analogs that can be substituted, from Aldrich, Milwaukee, WI, platinum on carbon, and palladium on carbon. Further examples include ferredoxin molecules of appropriate oxidation/reduction potential, such as the ferredoxin formed of rubredoxin and other ferredoxins available from Sigma Chemical. Other electron transfer mediators include organic compounds such as quinone and related compounds. The electron  
15   transfer mediator can be applied, for example, by screening or masked dip coating or sublimation. The first electrode can be impregnated with the redox enzyme, which can be applied before or after the electron transfer mediator. One way to assure the association of the redox enzyme with the electrode is simply to incubate a solution of the redox enzyme with electrode for sufficient time to allow associations between the electrode and the enzyme, such  
20   as Van der Waals associations, to mature. Alternatively, a first binding moiety, such as biotin or its binding complement avidin/streptavidin, can be attached to the electrode and the enzyme bound to the first binding moiety through an attached molecule of the binding complement.

          The redox enzyme can comprise any number of enzymes that use an electron carrier as  
25   a substrate, irrespective of whether the primary biologically relevant direction of reaction is for the consumption or production of such reduced electron carrier, since such reactions can be conducted in the reverse direction. Examples of redox enzymes further include, without limitation, glucose oxidase (using NADH, available from several sources, including number of types of this enzyme available from Sigma Chemical), glucose-6-phosphate dehydrogenase  
30   (NADPH, Boehringer Mannheim, Indianapolis, IN), 6-phosphogluconate dehydrogenase (NADPH, Boehringer Mannheim), malate dehydrogenase (NADH, Boehringer Mannheim), glyceraldehyde-3-phosphate dehydrogenase (NADH, Sigma, Boehringer Mannheim),

isocitrate dehydrogenase (NADH, Boehringer Mannheim; NADPH, Sigma), and  $\alpha$ -ketoglutarate dehydrogenase complex (NADH, Sigma).

The redox enzyme can also be a transmembrane pump, such as a proton pump, that operates using an electron carrier as the energy source. In this case, enzyme can be associated  
5 with the electrode in the presence of detergent and/or lipid carrier molecules which stabilize the active conformation of the enzyme. As in other embodiments, an electron transfer mediator can be used to increase the efficiency of electron transfer to the electrode.

Associated electron carriers are readily available from commercial suppliers such as Sigma and Boehringer Mannheim. The concentrations at which the reduced form of such  
10 electron carriers can be as high as possible without disrupting the function of the redox enzyme. The salt and buffer conditions are designed based on, as a starting point, the ample available knowledge of appropriate conditions for the redox enzyme. Such enzyme conditions are typically available, for example, from suppliers of such enzymes.

As illustrated for the fuel cell **100** in **Figure 4A** (top view), a source reservoir **111** can  
15 be provided to supply reduced electron carrier via conduit **113**, check-valve **112** and diffuser **114** to second chamber **102**. Note that fuel cell **100** lacks a first chamber as this chamber often serves as a reservoir, which in fuel cell **100** is provided by source reservoir **111**. Diffuser **115**, conduit **116**, and pump **117** provide the pathway and motive power for conveying spent liquid containing the electron carrier (often merely having reduced  
20 effectiveness in powering the fuel cell) to an output reservoir **118**. Fuel cell **100** further has a first electrode **104**, second electrode **105**, third chamber **103**, air pump **121**, air inlet **122**, and air outlet **123**. The various pumps can be operated off of a battery, which can be recharged and regulated using energy from the fuel cell, or can come into operation after the fuel cell begins generating current. As illustrated in **Figure 4B**, voltage or current monitor **M** can  
25 monitor the performance the fuel cell in providing voltage to the circuit comprising resistor(s) **R**. Monitor **M** can relay information to the controller, which uses the information to regulate operation of one or more of the pumps.

**Figure 5A** illustrates a fuel cell **200** (top view) in which an acid/base reservoir **231** serves to supply a source of a material required to account for any material imbalances in the  
30 reaction equations at the first and second electrodes. The acid/base reservoir **231** is connected via conduit **232**, first actuated valve **233**, and diffuser **234** to a second chamber **202**. Liquid from source reservoir **211** is delivered via check valve **212A** and second actuated valve **212B**.

In one example of operation, second actuated valve **212B** is normally open, and first actuated valve **233** is normally closed. These valve positions are reversed when the controller detects the need for fluid from acid/base reservoir **231** (e.g., because of a signal received from a pH monitor) and operates pump **117** (e.g., by use of a stepper motor) to draw fluid into the  
5 second chamber **202**.

It will be recognized that the pump and valve arrangements in **Figures 4A** through **5B** are for illustration only, as numerous alternative arrangements will be recognized by those of ordinary skill. The plumbing of the fuel cell can be arranged to maintain a chamber less than atmospheric pressure, for instance to help reduce fluid leakage through various porous  
10 materials. The pores in various porous materials can be selected to allow such diffusion as is needed while minimizing fluid flow across the porous materials, such as bulk liquid flow into a chamber designed to bring gas into contact with a porous electrode.

The chambers of fluid which the first and second electrodes contact can be independent, as illustrated in **Figure 6**. In fuel cell **300**, the solution bathing the first  
15 electrode (anode) is fed through conduit **313A**, while that bathing the second electrode (cathode) is supplied through conduit **313B**. Flow is illustrated as regulated by pumps **317A** and **317B**. In the illustrated fuel cell, the bathing solutions are replenished as needed to account for the necessary imbalance in the chemistries occurring in the segregated cells.

Cells can be stacked, and electrodes arranged in a number of ways to increase the  
20 areas of contact between electrodes and reactants. These stacking and arranging geometries can be based on well-known geometries used with conventional fuel cells.

It will be recognized that where the electron carrier has an appropriate electrochemical potential relative to the electron-receiving molecule, the cell can be operated so that the *oxidized form* of the electron carrier receives the electrons through an enzyme catalyzed  
25 event. For example, the electron carrier and the electron-receiving molecule can both be of the class exemplified for electron carriers, but with distinct electrochemical potentials. Thus, both the fuel side and product side reactions can be enzyme catalyzed. In fact, even with such traditional electron-receiving composition as oxygen, the product side reaction can be enzyme catalyzed.

30 In one embodiment of the invention, the fuel cell does not incorporate a proton pump. Preferably, in this embodiment the redox enzyme is associated with a lipid component, such as a composition containing phospholipid, steroids (such as sterols), glycolipids,

sphingolipids, triglyceride or other components typically incorporated into intracellular or external cellular membranes, while still being sufficiently associated with the electrodes to convey electrons. The enzyme is preferably incorporated into a lipid bilayer. The barrier can be separating component such as is used in a typical fuel cell, which preferably conveys  
5 protons between the first and second chambers, though without requiring proton pumping.

The following examples further illustrate the present invention, but of course, should not be construed as in any way limiting its scope.

#### Example

The test apparatus consisted of a 5 ml reaction vessel which held the fuel and into  
10 which copper or other electrodes were dipped. The electrodes were in turn connected to a high impedance voltmeter for open circuit voltage measurements or to a low impedance ammeter for short circuit current measurements. Various test configurations were employed to establish a baseline with which to measure performance of the cell. Testing was done by dipping electrodes in the fuel solution and measuring current and / or voltage as a function of  
15 time.

The reaction which drove the cell was the oxidation of nicotinamide-adenine dinucleotide hydride (NADH) which is catalyzed by the enzyme glucose oxidase (GOD) in the presence of glucose. This reaction yielded  $\text{NAD}^+$ , a proton ( $\text{H}^+$ ) and 2 free electrons.



20 The reaction took place at one electrode, which was a metallized plastic strip coated with the enzyme GOD. This half-reaction was coupled through an external circuit to the formation of water or hydrogen peroxide from protons, dissolved oxygen, and free electrons at the other electrode.

Fuels used were solutions of glucose, NADH or combinations thereof, distilled  
25 deionized water or a 50 mM solution of Tris<sup>TM</sup> 7.4 buffer. (NADH is most stable in a pH 7.4 environment.) Electrode materials were copper (as a reference) and metallized plastic strips coated with GOD (a commercially available product).

Test configurations employed as well as initial results were as follows:

#### **Configuration 1:**

30 Electrode 1: Copper  
Electrode 2: Copper  
Solution: 50 mM tris 7.4 buffer



Voltage: -7.5 mV

Current: 3  $\mu$ A initially decaying to -2.2  $\mu$ A within 3 minutes, fairly constant thereafter.

**Configuration 2:**

5        Electrode 1: Copper  
         Electrode 2: GOD coated strip  
         Solution: 50 mM tris 7.4 buffer  
         Voltage: + 350 mV  
         Current: > 20  $\mu$ A (+) initially decaying to + 4  $\mu$ A within 2 minutes, fairly constant  
10        thereafter.

**Configuration 3:**

         Electrode 1: Copper  
         Electrode 2: Copper  
         Solution: 10 mM glucose in 50 mM tris 7.4 buffer  
15        Voltage: -6.3 mV Current: -1.7  $\mu$ A, fairly constant after initial dropoff.

**Configuration 4:**

         Electrode 1: Copper  
         Electrode 2: GOD coated strip  
         Solution: 10 mM glucose in 50 mM tris 7.4 buffer  
20        Voltage: + 350 mV  
         Current: > 20  $\mu$ A (+) initially decaying to ~ + 2  $\mu$ A within 2 minutes, fairly constant thereafter.

**Configuration 5:**

         Electrode 1: Copper  
25        Electrode 2: Copper  
         Solution: 10 mM glucose + 10 mM NADH in 50 mM tris 7.4 buffer  
         Voltage: -290 mV slowly increasing to - 320 after 4 minutes  
         Current: -25  $\mu$ A, decaying to -21  $\mu$ A after 2 minutes.

**Configuration 6:**

30        Electrode 1: Copper  
         Electrode 2: GOD coated strip  
         Solution: 10 mM glucose + 10 mM NADH in 50 mM tris 7.4 buffer

Voltage: + 500 mV decaying to +380 after 2 minutes

Current: > + 30  $\mu$ A, dropping rapidly to ~ + 1  $\mu$ A after 1 minute.

All publications and references, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference in their entirety as if each individual publication or reference were specifically and individually indicated to be incorporated by reference herein as being fully set forth. Any patent application to which this application claims priority is also incorporated by reference herein in its entirety in the manner described above for publications and references.

While this invention has been described with an emphasis upon preferred embodiments, it will be obvious to those of ordinary skill in the art that variations in the preferred devices and methods may be used and that it is intended that the invention may be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the invention as defined by the claims that follow.

What is claimed:

1. A battery comprising a first compartment, a second compartment and a barrier separating the first and second compartments, wherein the barrier comprises a proton transporting moiety.

5

2. A battery comprising:  
a first compartment;  
a second compartment;  
a barrier separating the first compartment from the second compartment; said barrier  
10 having a proton transporting moiety;  
a first electrode;  
a second electrode;  
a redox enzyme in the first compartment in communication with the first electrode to receive electrons therefrom;  
15 an electron carrier in the first compartment in chemical communication with the redox enzyme; and  
an electron receiving composition in the second compartment in chemical communication with the second electrode,  
wherein, in operation, an electrical current flows along a conductive pathway formed between  
20 the first electrode and the second electrode.

3. The battery of claim 2, wherein the first electrode is further associated with an electron transfer mediator that transfers electrons from the redox enzyme to the first electrode.

25 4. The battery of claim 2, wherein the proton transporting protein comprises at least a portion of the redox enzyme.

5. The battery of claim 2, further comprising a reservoir for supplying to the vicinity of at least one of the electrodes a component consumed in the operation of the battery and a  
30 pump for drawing such component to that vicinity.

6. The battery of claim 5, further comprising a controller which receives data on the operation of the battery and controls the pump in response to the data.

7. The battery of claim 2, wherein a light-driven proton pump protein comprises at least  
5 a portion of the proton transporting protein, and further comprising:  
a source of light for powering the light-driven proton pump protein.

8. The battery of claim 2, further incorporating in the barrier a second protein, distinct  
from the first, adapted to facilitate reverse proton pumping when the battery is operated in  
10 recharge mode.

9. A method of operating a battery with a first compartment and a second compartment  
comprising:

enzymatically oxidizing an electron carrier and delivering the electrons to a  
15 first electrode in chemical communication with the first compartment;  
catalyzing the transfer of protons from the first compartment to the second  
compartment; and  
reducing an electron receiving molecule with electrodes conveyed through a  
circuit from the first electrode to a second electrode located in the  
20 second compartment.

10. The method of claim 9, wherein the catalytic transfer of protons occurs in conjunction  
with the enzymatic oxidation of the electron carrier.

25 11. The method of claim 9, wherein at least a portion of the transfer of protons is driven  
by a light-driven proton pump protein, and the method further comprises:  
directing light to the light-driven proton pump.

12. The method of claim 11, further comprising  
30 monitoring the pH of the first compartment and controlling the amount of light  
directed to the light-driven proton pump such that relatively more light  
is directed at lower pH values.

13. The method of claim 9, further comprising:

applying a voltage to the electrodes of a polarity opposite that generated by the normal operation of the battery to recharge the battery.

5

14. The method of claim 13, further comprising:

enzymatically transporting protons from the second chamber to the first chamber in connection with the applying the recharge voltage.

10 15. The method of claim 14, wherein at least a portion of the enzymatic transport in recharge mode is accomplished by an enzyme distinct from an enzyme catalyzing the majority of proton transport in a power producing mode.

16. A battery comprising:

15

a first compartment;

a second compartment;

a barrier separating the first compartment from the second compartment;

a first electrode;

a second electrode;

20

a redox enzyme in the first compartment in communication with the first electrode to receive electrons therefrom, the redox enzyme incorporated in a lipid composition;

an electron carrier in the first compartment in chemical communication with the redox enzyme; and

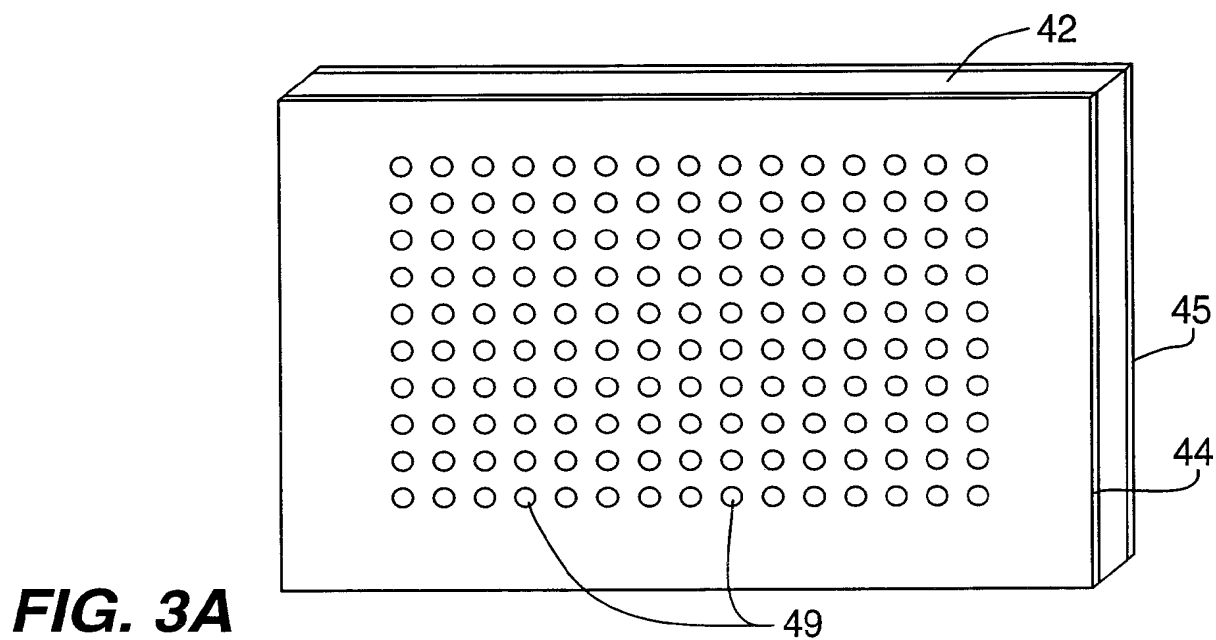
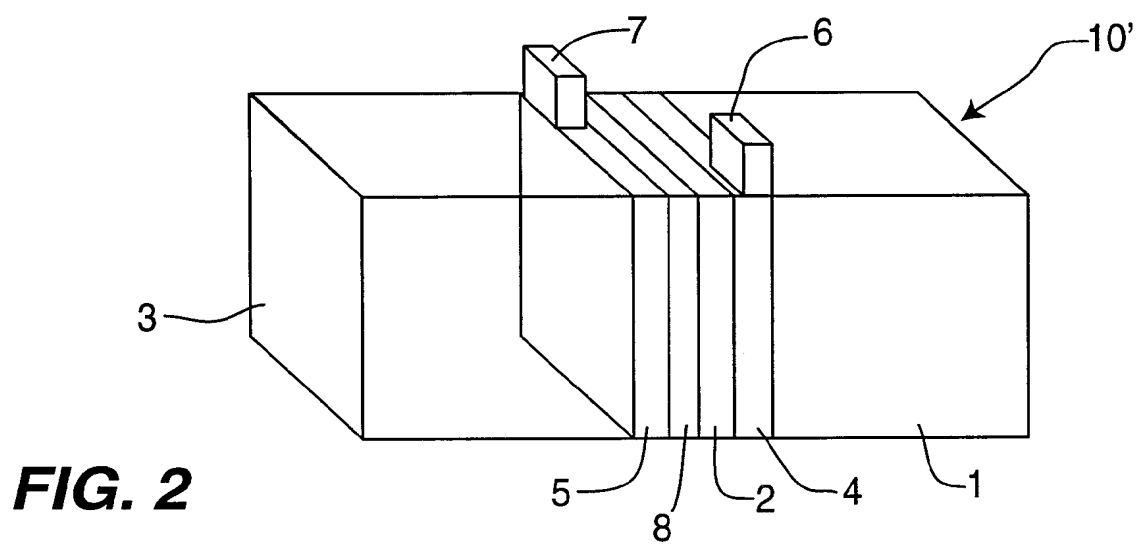
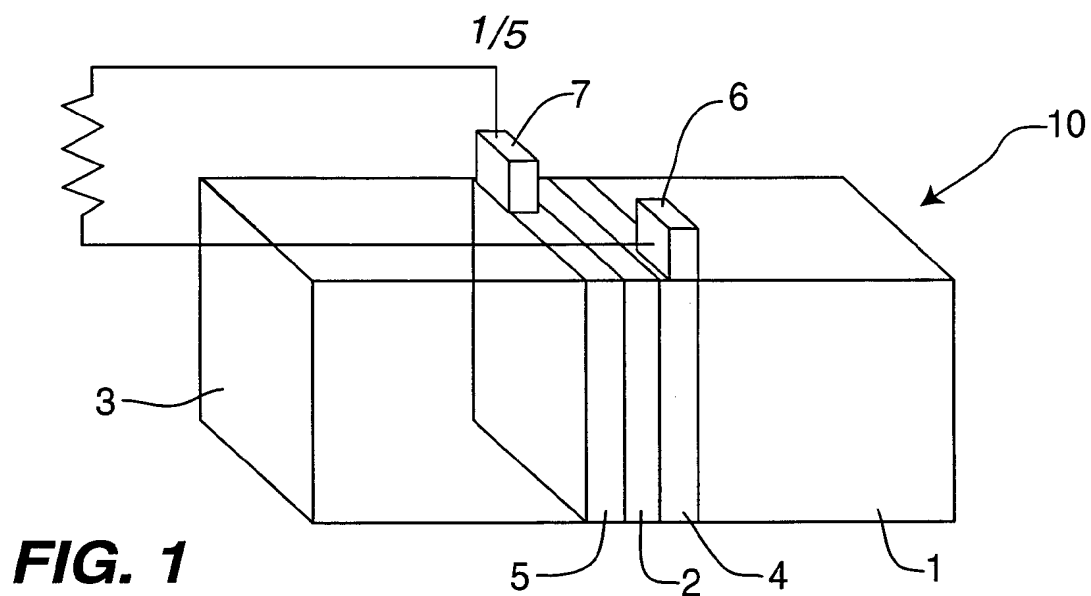
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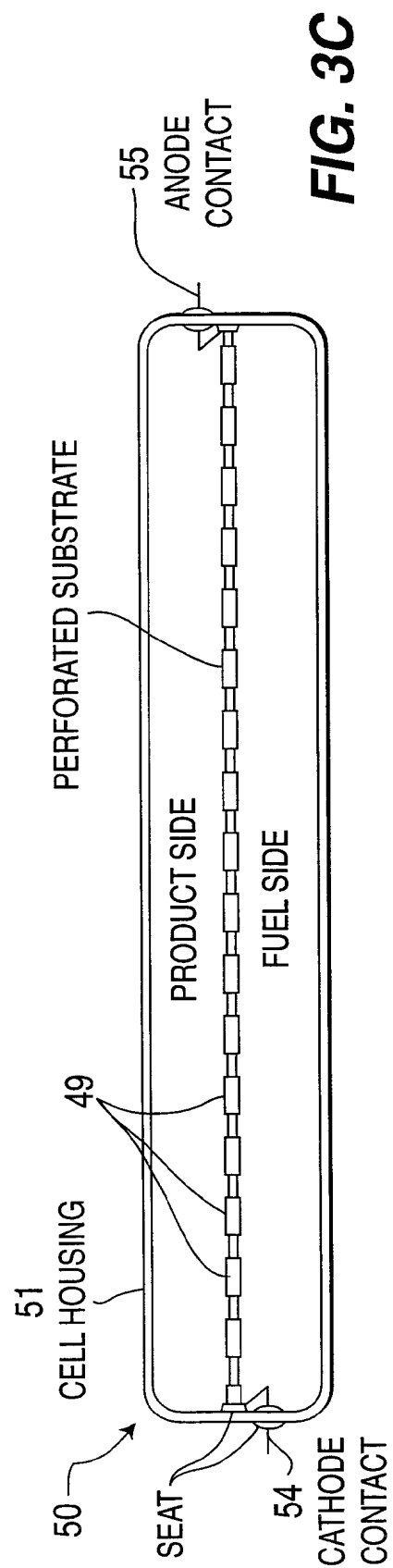
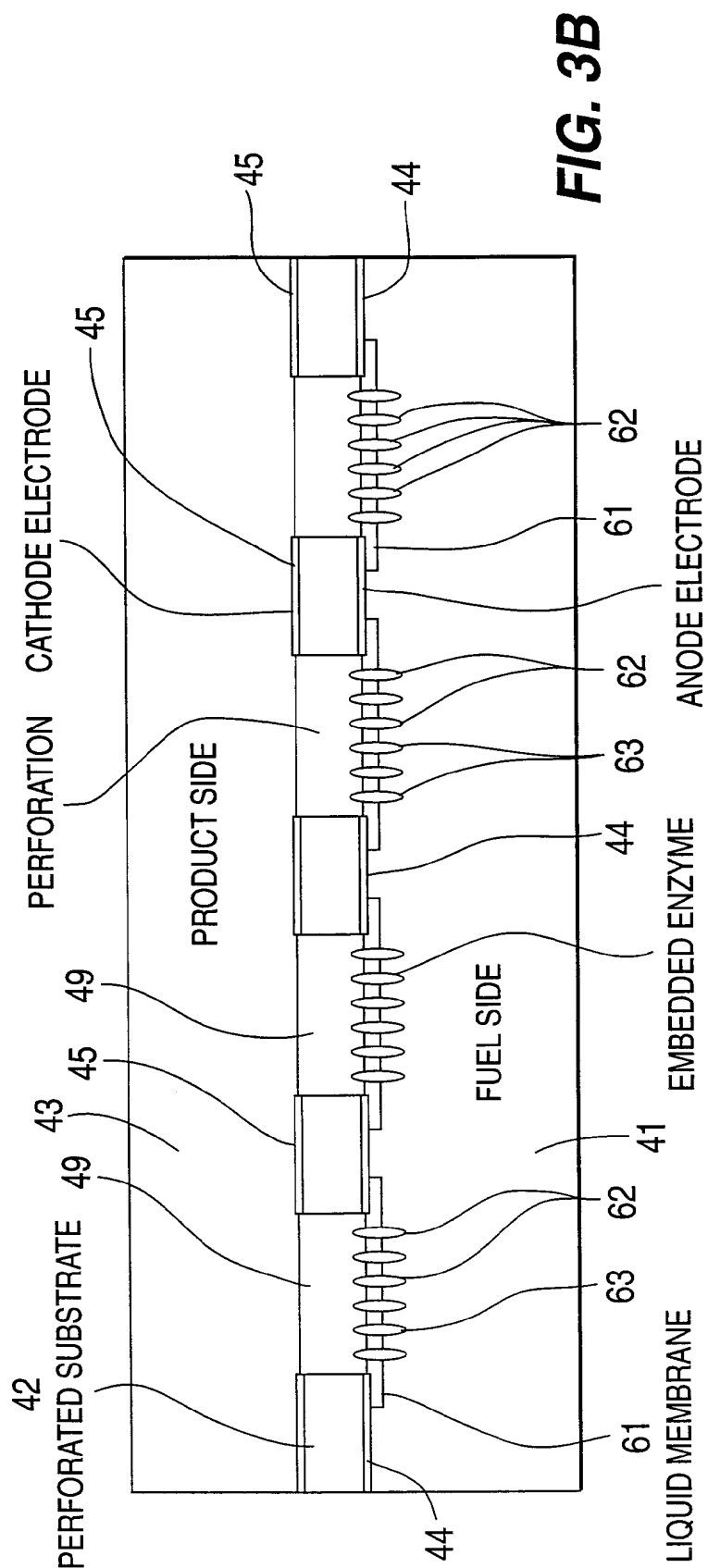
an electron receiving composition in the second compartment in chemical communication with the second electrode,

wherein, in operation, an electrical current flows along a conductive pathway formed between the first electrode and the second electrode.

17. A method of operating a battery with a first compartment and a second compartment comprising:

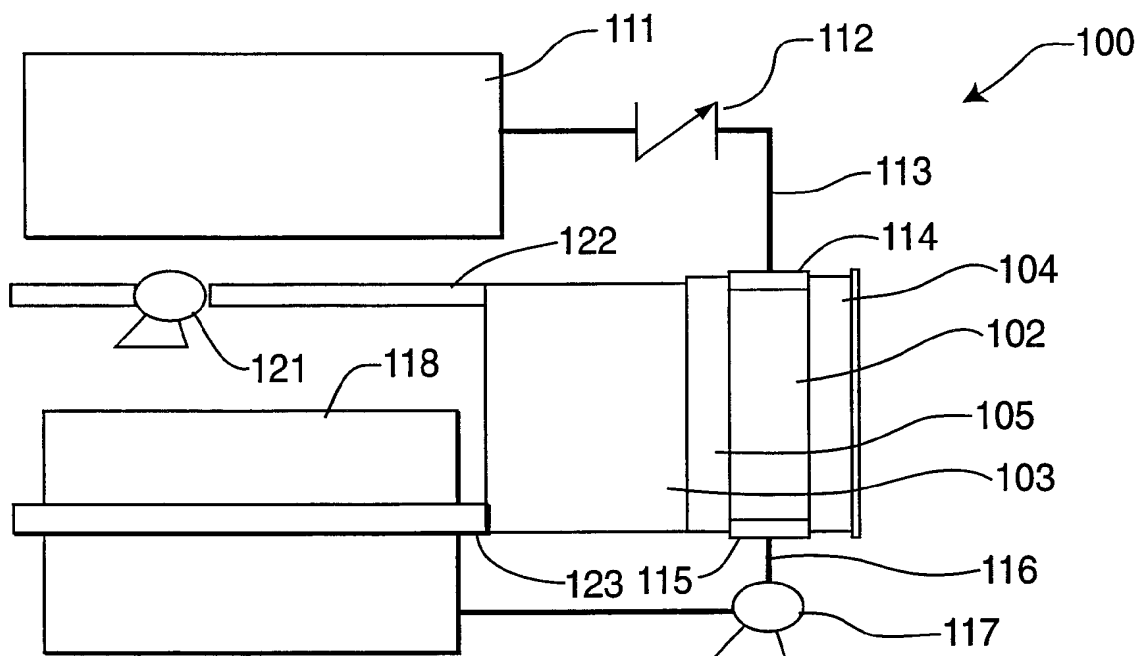
- 5 enzymatically oxidizing, with an enzyme incorporated into a lipid composition, an electron carrier and delivering the electrons to a first electrode in chemical communication with the first compartment; and
- reducing an electron receiving molecule with electrodes conveyed through a circuit from the first electrode to a second electrode located in the second compartment.



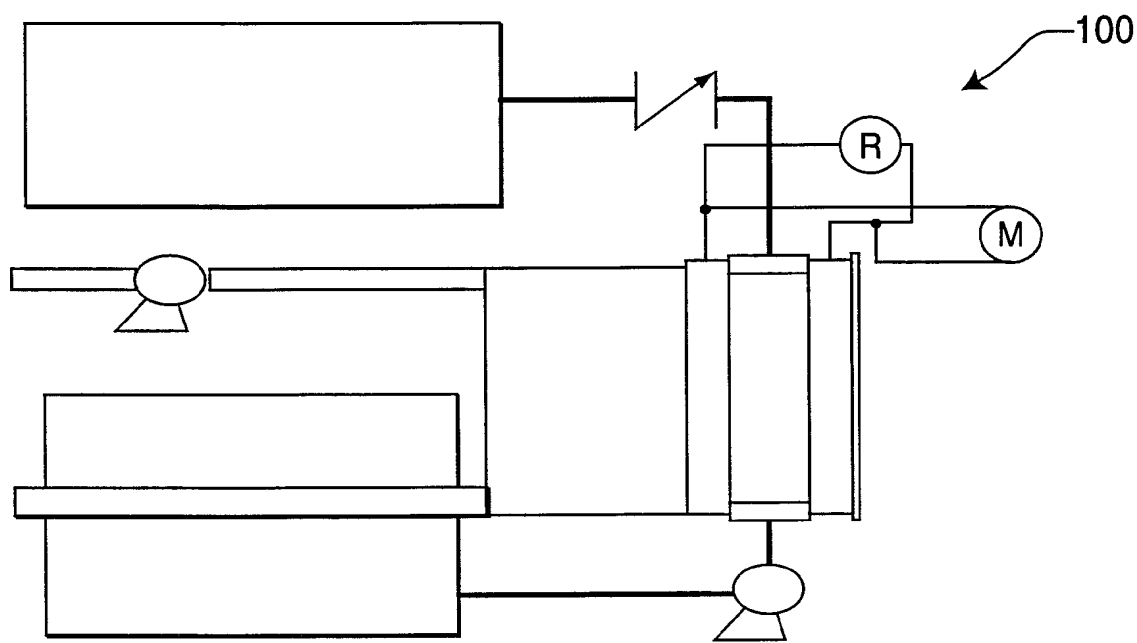




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**FIG. 4A**



**FIG. 4B**

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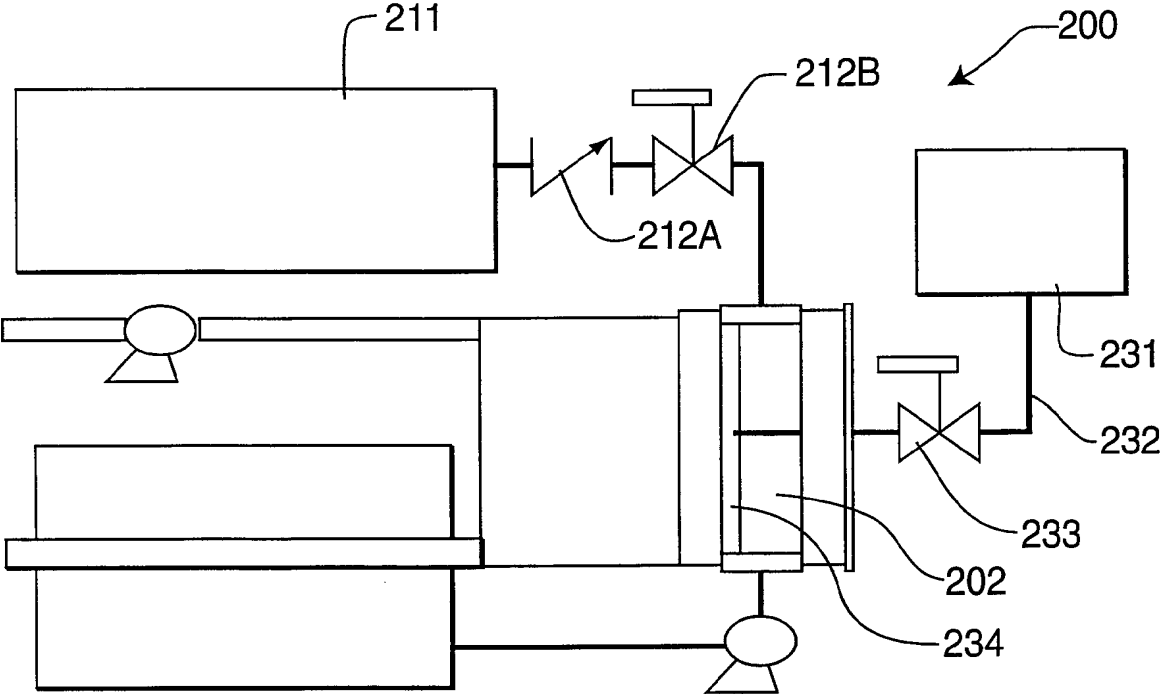


FIG. 5A

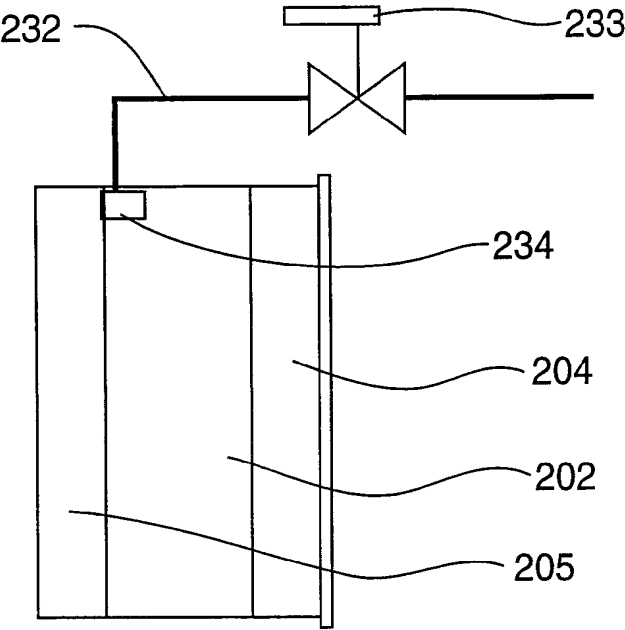
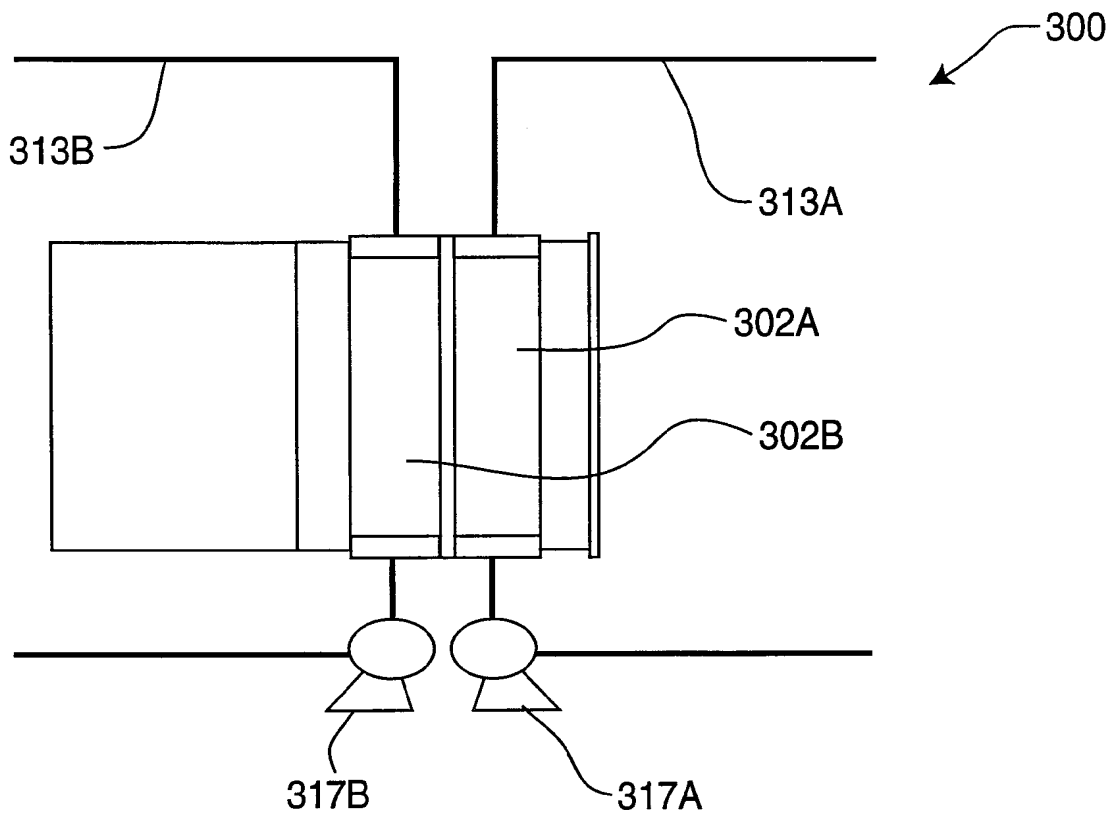
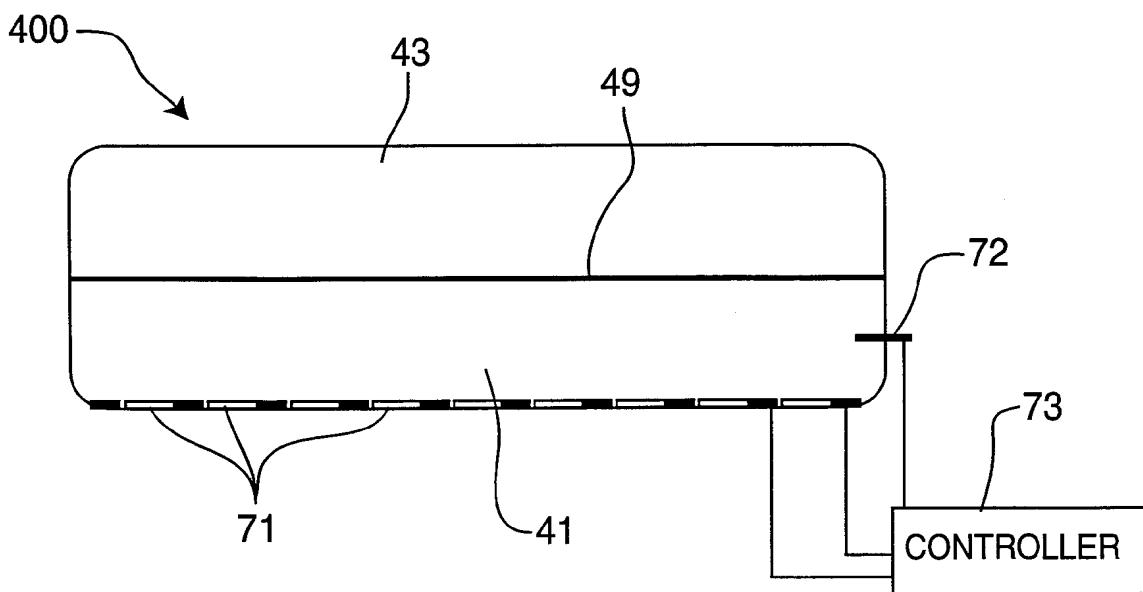


FIG. 5B

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**FIG. 6**



**FIG. 7**

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/18804

A. CLASSIFICATION OF SUBJECT MATTER  
 IPC 7 H01M8/16 H01M14/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 H01M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data, WPI Data, PAJ, BIOSIS, INSPEC

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BOCKRIS J O'M ET AL: "A FUEL CELL MODEL IN BIOLOGICAL ENERGY CONVERSION" JOURNAL OF BIOLOGICAL PHYSICS, NL, KLUWER ACADEMIC PUBLISHERS, DORDRECHT, vol. 13, no. 1, 1985, pages 3-12, XP000905113 ISSN: 0092-0606 the whole document	1-16
A	MITCHELL P: "PROTON-TRANSLOCATION PHOSPHORYLATION IN MITOCHONDRIA, CHLOROPLASTS AND BACTERERIA: NATURAL FUEL CELLS AND SOLAR CELLS" FEDERATION PROCEEDINGS, vol. 26, no. 5, October 1967 (1967-10), pages 1370-1379, XP000905259 the whole document	1-16



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

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Date of the actual completion of the international search

12 July 2000

Date of mailing of the international search report

19/07/2000

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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/18804

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CRUNDWELL F K: "THE KINETICS OF THE CHEMIOSMOTIC PROTON CIRCUIT OF THE IRON-OXIDIZING BACTERIUM THIOBACILLUS FERROOXIDANS" BIOELECTROCHEMISTRY AND BIOENERGETICS, XX, XX, vol. 43, no. 1, 1997, pages 115-122, XP000905141 ISSN: 0302-4598 the whole document ----	1-16
A	AHN B K ET AL: "A SEPHAROSE MEMBRANE BOUND HYALURONIDASE TO PROCESS EXTRA-CELLULAR FLUID FOR THE IMPLANTABLE BIOAUTOFUEL CELL" PROCEEDINGS ANNUAL CONFERENCE ENGINEERING IN MEDICINE AND BIOLOGY , 6 October 1974 (1974-10-06), page 200 XP000923092 Philadelphia, Pennsylvania, USA the whole document ----	1-16
A	DE 44 44 893 A (MERCK PATENT GMBH) 20 June 1996 (1996-06-20) page 6, line 26 -page 8, line 7 -----	1-16

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/18804

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE 4444893 A	20-06-1996	AU 4257596 A	03-07-1996
		WO 9618645 A	20-06-1996
		EP 0800529 A	15-10-1997
		JP 10510277 T	06-10-1998
		US 5962638 A	05-10-1999

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